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(54) Title: NOVEL ORPHAN RECEPTOR

(57) Abstract

The present invention provides for a nucleic acid sequence, designated as HUMAN NTR-1, that encodes a novel orphan receptor expressed in pancreas and fetal heart. The invention also provides assay systems that may be used to detect and/or measure ligands that bind the HUMAN NTR-1 gene product. The present invention also provides for diagnostic and therapeutic methods based on the interaction between NTR-1 and agents that initiate signal transduction through binding to NTR-1.

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NOVEL ORPHAN RECEPTOR

This International Application claims priority of U.S. Provisional Application Serial No. 60/054,869 filed August 6, 1997. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INTRODUCTION

The field of this invention is polypeptide molecules which regulate cell function, nucleic acid sequences encoding the polypeptides, and methods of using the nucleic acid sequences and the polypeptides. The present invention provides for novel orphan receptor molecules, their use and assay systems useful for identifying novel ligands that interact with these receptors.

BACKGROUND OF THE INVENTION

The tumor necrosis factor receptor (TNFR) superfamily consists mostly of transmembrane proteins that elicit signal transduction in a variety of cells. Tumor necrosis factor-alpha (TNF-alpha) is a cytokine primarily produced by activated macrophages. TNF-alpha stimulates T-cell and B-cell proliferation and induces expression of adhesion molecules on endothelial cells. This cytokine also plays an important role in host defense to infection.

TNF-alpha activities are mediated through two distinct receptors, TNFR-p55 and TNFR-p75. These two receptors also mediate activities triggered by soluble lymphotoxin-alpha (LT-alpha) secreted mainly by activated lymphocytes. Specific stimulation of TNFR-p55 induces TNF activities such

as in vitro tumor cell cytotoxicity, expression of adhesion molecules on endothelial cells and keratinocytes, activation of sphingomyelinase with concomitant increases of ceramide, activation of NF-kappaB and induction of manganese superoxide dismutase mRNA. Specific stimulation of TNFR-p75 results in proliferative response of mouse and human thymocytes and cytotoxic T cells, fibroblasts and natural killer cells and in GM-CSF secretion in PC60 cells.

The identification of a new member of the TNFR superfamily that regulates bone resorption was recently reported. The newly identified protein was termed Osteoprotegerin (OPG) and was postulated to act as a humoral regulator of bone resorption by blocking the differentiation of osteoclasts, the cells responsible for bone resorption. (Simonet, W.S., et al., 1997, Cell 89: 309-319; International Publication Number WO 97/ 23614 published 3 July 1997 in the name of Amgen, Inc.). However, relatively little is known about the soluble factors that act physiologically to regulate osteoclast development.

Novel receptor molecules are often identified and isolated by searching for additional members of known families of receptors using, for example, PCR-based screens or computer searches of EST databases involving known regions of homology among the family members. (See, for example, Maisonpierre, et al., 1993, Oncogene 8: 1631-1637). Isolation of such so called "orphan" receptors, for which no ligand is known, and subsequent determination of the tissues in which such receptors are expressed, provides insight into the regulation of the growth, proliferation and regeneration of cells in target tissues. Further, such receptors may be used to isolate their cognate ligands, which may then be used to regulate the survival, growth and regeneration of cells expressing the receptor. Alternatively, in the case of soluble receptors, the receptor itself can behave as a ligand.

SUMMARY OF THE INVENTION

The present invention provides for a novel orphan human receptor, termed HUMAN NTR-1, which is expressed in the pancreas, skeletal muscle, and fetal and adult hearts. The protein is related to osteoprotegerin (OPG) and to tumor necrosis factor receptor (TNFR). The present invention further provides for an isolated nucleic acid molecule encoding HUMAN NTR-1. Based upon its homology to osteoprotegerin, it is expected that HUMAN NTR-1 will be involved in the regulation of bone mass, and may be useful for regulating development, proliferation and death of osteoblast or osteoclast cells or for regulating muscle metabolism and may be implicated in diseases or disorders of muscle.

The present invention also provides for a protein or polypeptide that comprises the extracellular domain of HUMAN NTR-1 as well as the nucleic acid which encodes such extracellular domain. The invention further provides for vectors comprising an isolated nucleic acid molecule encoding HUMAN NTR-1 or its extracellular domain, which can be used to express HUMAN NTR-1 or its extracellular domain in bacteria, yeast, insect or mammalian cells, preferably COS or CHO cells.

The invention further provides for use of the HUMAN NTR-1 receptor or its extracellular or intracellular domain in screening for drugs that interact with HUMAN NTR-1. Novel agents that bind to the receptor(s) described herein may mediate survival and differentiation in cells naturally expressing the receptor, but also may confer survival and proliferation when used to treat cells engineered to express the receptor. In particular embodiments, the extracellular domain (soluble receptor) of HUMAN NTR-1 is utilized in screens for cognate ligands.

Preferred uses for the subject HUMAN NTR-1 polypeptides include screening for agents that bind to the receptor polypeptides. The agents may be biologically active agents (agonists), which activate the HUMAN NTR-1 receptor or they may bind and block activation of the receptor (antagonists). Screening methods include incubating a HUMAN NTR-1 polypeptide in the presence of an extracellular HUMAN NTR-1 polypeptide-specific binding target and a candidate agent under conditions whereby, but for the presence of the agent, the polypeptide specifically binds the binding target at a reference affinity; detecting the binding affinity of the polypeptide to the binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that the agent modulates the binding of the polypeptide to the binding target.

The invention also provides for a nucleic acid probe capable of hybridizing with a sequence included within the nucleic acid sequence encoding HUMAN NTR-1 useful for the detection of NTR-1 expressing tissue in humans and animals.

The invention further provides for antibodies directed to HUMAN NTR-1.

The present invention also has diagnostic and therapeutic utilities. In particular embodiments of the invention, methods of detecting aberrancies in the function or expression of the receptor described herein may be used in the diagnosis of disorders. In other embodiments, manipulation of the receptor or agonists or antagonists which bind this receptor may be used in the treatment of diseases. In further embodiments, the extracellular domain of the receptor is utilized as a blocking agent which blocks the binding of receptor to its target.

In a further embodiment of the invention, patients who suffer from an excess of NTR-1 may be treated by administering an effective amount of anti-sense RNA or anti-sense oligodeoxyribonucleotides corresponding to the HUMAN NTR-1 gene coding region, thereby decreasing expression of NTR-1.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides HUMAN NTR-1 polypeptide which includes isolated HUMAN NTR-1 polypeptide and recombinant polypeptides comprising a HUMAN NTR-1 amino acid sequence, or a functional HUMAN NTR-1 polypeptide domain thereof having an assay-discernable HUMAN NTR-1-specific activity. Accordingly, the polypeptides may be deletion mutants of the disclosed HUMAN NTR-1 polypeptides and may be provided as fusion products, e.g., with non - HUMAN NTR-1 polypeptides. The subject HUMAN NTR-1 polypeptide domains have HUMAN NTR-1-specific activity or function.

A number of applications for HUMAN NTR-1 are suggested from its properties. HUMAN NTR-1, may be useful in the study and treatment of conditions similar to those which are treated using TNF. Furthermore, the HUMAN NTR-1 cDNA may be useful as a diagnostic tool, such as through use of antibodies in assays for polypeptides in cell lines or use of oligonucleotides as primers in a PCR test to amplify those with sequence similarities to the oligonucleotide primer, and to see how much HUMAN NTR-1 is present. The isolation of HUMAN NTR-1, of course, also provides the key to isolate its putative ligand, other HUMAN NTR-1 binding polypeptides, and/or study its properties.

HUMAN NTR-1-specific activity or function may be determined by convenient in vitro, cell based, or in vivo assays - e.g., in vitro binding assays, cell culture assays, in animals (e.g., immune response, gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the specific molecular interaction of a HUMAN NTR-1 polypeptide with a binding target is evaluated. The binding target may be a natural binding target, or a non-natural binding target such as a specific immune polypeptide such as an antibody, or a HUMAN NTR-1 specific agent such as those identified in assays described below.

The claimed polypeptides may be isolated or pure - an "isolated" polypeptide is one that is no longer accompanied by some of the material with which it is associated in its natural state, and that preferably constitutes at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample; a "pure" polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. The subject polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g., Molecular Cloning, A Laboratory Manual (Sambrook, et al., Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY).

The subject polypeptides find a wide variety of uses including use as immunogens, targets in screening assays, bioactive reagents for modulating cell growth, differentiation and/or function, etc. For example, the invention provides methods for modifying the physiology of a cell comprising an extracellular surface by contacting the cell or medium

surrounding the cell with an exogenous HUMAN NTR-1 polypeptide under conditions whereby the added polypeptide specifically interacts with a component of the medium and/or the extracellular surface to effect a change in the physiology of the cell. According to these methods, the extracellular surface includes plasma membrane-associated receptors; the exogenous HUMAN NTR-1 refers to a polypeptide not made by the cell or, if so, expressed at non-natural levels, times or physiologic locales; and suitable media include in vitro culture media and physiological fluids such as blood, synovial fluid, etc. The polypeptides may be may be introduced, expressed, or repressed in specific populations of cells by any convenient way such as microinjection, promoter-specific expression of recombinant enzyme, targeted delivery of lipid vesicles, etc.

The invention provides HUMAN NTR-1-specific binding agents, methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. HUMAN NTR-1-specific binding agents include HUMAN NTR-1-specific receptors, such as somatically recombined protein receptors like specific antibodies or T-cell antigen receptors (See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and also includes other binding agents identified with assays such as one-, two- and three-hybrid screens, and non-natural binding agents identified in screens of chemical libraries such as described below. Agents of particular interest modulate HUMAN NTR-1 function.

The invention provides HUMAN NTR-1 nucleic acids, which find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc., as well as use in detecting the presence of HUMAN NTR-1 genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional HUMAN NTR-1 homologs and structural analogs.

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e., no longer accompanied by some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Nucleic acids comprising the nucleotide sequence disclosed herein and fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by a sequence other than that to which it is joined on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which is immediately flanked by a sequence other than that to which it is joined on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The amino acid sequences of the disclosed HUMAN NTR-1 polypeptide is used to back translate HUMAN NTR-1 polypeptide-encoding nucleic acids optimized for selected expression systems (Holler, et al. (1993) Gene 136: 323-328; Martin, et al. (1995) Gene 154: 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural HUMAN NTR-1 encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc., Madison, WI). HUMAN NTR-1 encoding nucleic acids may be part of expression vectors and may be incorporated into recombinant host cells, e.g., for expression and screening, for transgenic animals, for functional studies such as the efficacy of candidate drugs for disease associated with HUMAN NTR-1 mediated signal transduction, etc. Expression systems are selected and/or tailored to effect HUMAN NTR-1 polypeptide structural and functional variants through alternative post-translational processing.

The invention also provides for nucleic acid hybridization probes and replication/amplification primers having a HUMAN NTR-1 cDNA specific sequence and sufficient to effect specific hybridization with SEQ. NO. 1. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M $NaPO_4$, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSPE buffer at 42°C. HUMAN NTR-1 cDNA homologs can also be distinguished from other polypeptides using alignment algorithms, such as BLASTX (Altschul, et al. (1990) Basic Local Alignment Search Tool, J. Mol. Biol. 215: 403-410).

HUMAN NTR-1 hybridization probes find use in identifying wild-type and mutant alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. HUMAN NTR-1 nucleic acids are also used to modulate cellular expression or intracellular concentration or availability of active HUMAN NTR-1. HUMAN NTR-1 inhibitory nucleic acids are typically antisense - single stranded sequences comprising complements of the disclosed HUMAN NTR-1 coding sequences. Antisense modulation of the expression of a given HUMAN NTR-1 polypeptide may employ antisense nucleic acids operably linked to gene regulatory sequences. Cells are transfected with a vector comprising a HUMAN NTR-1 sequence with a promoter sequence oriented such that transcription of the gene yields an antisense transcript capable of binding to endogenous HUMAN NTR-1 encoding mRNA. Transcription of the antisense nucleic acid may be constitutive or inducible and the vector may provide for stable extrachromosomal maintenance or integration. Alternatively, single-

stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given HUMAN NTR-1 polypeptide may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results in a substantial reduction in expression of the targeted polypeptide. An enhancement in HUMAN NTR-1 expression is effected by introducing into the targeted cell type HUMAN NTR-1 nucleic acids which increase the functional expression of the corresponding gene products. Such nucleic acids may be HUMAN NTR-1 expression vectors, vectors which upregulate the functional expression of an endogenous allele, or replacement vectors for targeted correction of mutant alleles. Techniques for introducing the nucleic acids into viable cells are known in the art and include retroviral-based transfection, viral coat protein-liposome mediated transfection, etc.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of HUMAN NTR-1 modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate the interaction of HUMAN NTR-1 with a natural HUMAN NTR-1 binding target. A wide variety of assays for binding agents are provided including protein-protein binding assays, immunoassays, cell based assays, etc. Preferred methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds.

In vitro binding assays employ a mixture of components including a HUMAN NTR-1 polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g., a tag for detection or anchoring, etc. The assay mixtures comprise a natural HUMAN NTR-1 binding target. While native binding targets may be used, it is frequently preferred to use portions thereof as long as the portion provides binding affinity and avidity to the subject HUMAN NTR-1 conveniently measurable in the assay. The

assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds, preferably small organic compounds, and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents such as salts, buffers, neutral proteins, e.g., albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., may also be included. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. The mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the HUMAN NTR-1 specifically binds the cellular binding target, portion or analog with a reference binding affinity. Incubation periods are chosen for optimal binding but are also minimized to facilitate rapid, high throughput screening.

After incubation, the agent-biased binding between the HUMAN NTR-1 and one or more binding targets is detected by any convenient way. For cell-free binding type assays, a separation step is often used to separate bound from unbound components. Separation may be effected by precipitation, immobilization, etc., followed by washing by, e.g., membrane filtration or gel chromatography. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection as radioactivity, luminescence, optical or electron density, etc., or indirect detection such as an epitope tag, an enzyme, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g., through optical or electron density, radiative emissions, nonradiative energy transfers, or indirectly detected with antibody conjugates, etc. A difference in the binding affinity of the HUMAN NTR-1 polypeptide to the target in the absence of the agent as

compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the HUMAN NTR-1 polypeptide to the corresponding binding target. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

The invention provides for a method for modifying the physiology of a cell comprising an extracellular surface in contact with a medium, said method comprising the step of contacting said medium with an exogenous HUMAN NTR-1 polypeptide under conditions whereby said polypeptide specifically interacts with at least one of a component of said medium and said extracellular surface to effect a change in the physiology of said cell.

The invention further provides for a method for screening for biologically active agents, said method comprising the steps of a) incubating a HUMAN NTR-1 polypeptide in the presence of an extracellular HUMAN NTR-1 polypeptide specific binding target and a candidate agent, under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity; b) detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

One embodiment of the invention is an isolated HUMAN NTR-1 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having HUMAN NTR-1-specific activity.

Another embodiment of the invention is a recombinant nucleic acid encoding HUMAN NTR-1 polypeptide comprising the amino acid sequence as set forth herein or a fragment thereof having HUMAN NTR-1-specific activity.

Still another embodiment is an isolated nucleic acid comprising a nucleotide sequence as set forth herein or a fragment thereof having at least 18 consecutive bases and sufficient to specifically hybridize with a nucleic acid having the sequence of set forth herein in the presence of natural HUMAN NTR-1 cDNA.

The present invention also provides for antibodies to the HUMAN NTR-1 polypeptide described herein which are useful for detection of the polypeptide in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward this HUMAN NTR-1 polypeptide, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

The monoclonal antibodies for diagnostic or therapeutic use may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody

molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of the HUMAN NTR-1 polypeptide described herein. For the production of antibody, various host animals can be immunized by injection with the HUMAN NTR-1 polypeptide, or a fragment or derivative thereof, including but not limited to rabbits, mice and rats. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum.

A molecular clone of an antibody to a selected HUMAN NTR-1 polypeptide epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$

fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

The following example is offered by way of illustration and not by way of limitation.

EXAMPLE 1: Cloning and Sequencing of HUMAN NTR-1 gene

Amino acid sequences of known human and mouse members of the TNF family were used as tblastn queries to search the NIH EST database of random fragments of mRNA sequences (Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-10). Each query generated a list of hits, i.e. EST sequences with a substantial sequence similarity to the query sequence. Typically, the hits on top of the list corresponded to mRNA copies of the query protein, followed by ESTs derived from other members of the family and random-chance similarities.

A parser program was used to combine and sort all the hits from searches with all the members of the family. This allowed rapid subtraction of all the hits corresponding to known proteins. The remaining hits were analyzed for conservation of sequence motifs characteristic for the family. Additional database searches were performed to identify overlapping ESTs. Two human cDNA clones, I.D. Nos. 366305 (the '305 clone) and 592256 (the '256 clone) from the I.M.A.G.E. consortium, were discerned to contain homologous sequence. These clones, GeneBank Accession Nos. AA025672 and AA155646, were obtained from Research Genetics, Inc. (Huntsville, AL)

and sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA).

The '305 clone contained a stop codon and poly-A tail and thus was determined to encode the 3' end of the molecule. The '256 clone aligned with the 5' end of the '305 clone over an approximately 300 nucleotide stretch. Together, the overlapping clones encode a polypeptide of approximately 220 amino acids, but was missing about 80 amino acids from the 5' end as compared to Osteoprotegerin. Further, since there was no coding sequence for a predicted signal peptide or for an initial methionine, the 5' end of the '256 clone was deemed incomplete. The 5' RACE procedure was then used to obtain a nucleotide sequence encoding 63 missing amino acids including the signal peptide. Using PCR primers flanking the coding sequence, a single fragment was constructed that contained the entire coding sequence. The sequence of HUMAN NTR-1 was then further confirmed by sequencing. The nucleotide and deduced amino acid sequence of HUMAN NTR-1 is set forth herein. Northern analysis revealed HUMAN NTR-1 transcript in adult lung, skeletal muscle, kidney, placenta and pancreas as well as in fetal heart and stomach.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to

those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding HUMAN NTR-1.

2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of:
   (a) the nucleotide sequence comprising the coding region of the HUMAN NTR-1 as set forth in SEQ. NO. 1;
   (b) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a) and which encodes a molecule having the biological activity of the HUMAN NTR-1; or
   (c) a nucleotide sequence which, but for the degeneracy of the genetic code would hybridize to a nucleotide sequence of (a) or (b), and which encodes a molecule having the biological activity of the HUMAN NTR-1.

3. A vector which comprises a nucleic acid molecule of claim 1 or 2.

4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.

5. A vector according to claim 3 or 4, which is a plasmid.

6. Isolated HUMAN NTR-1 polypeptide.

7. Isolated HUMAN NTR-1 polypeptide, having the amino acid sequence as set forth in SEQ. NO. 2.

8. A host-vector system for the production of HUMAN NTR-1 which comprises a vector of claim 3 or 4, in a host cell.

9. A host-vector system according to claim 8, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

10. A method of producing HUMAN NTR-1 which comprises growing cells of a host-vector system of claim 8 or 9, under condition NTR-1 so produced.

11. An antibody which specifically binds the HUMAN NTR-1 of claim 6 or 7.

12. An antibody according to claim 11, which is a monoclonal antibody.

13. A composition comprising HUMAN NTR-1 according to claim 6 or 7, and a carrier.

14. A composition comprising an antibody according to claim 11 or 12, and a carrier.

15. HUMAN NTR-1 according to claim 6 or 7, an antibody according to claim 11 or 12, or a composition according to claim 13 or 14, for use in a method of treatment of the human or animal body, or in a method of diagnosis.

16. A polypeptide produced by the method of claim 10.

17. A receptorbody comprising the extracellular portion of the HUMAN NTR-1 receptor fused to an immunoglobulin constant region.

18. The receptorbody of claim 17, wherein the constant region is the human immunoglobulin gamma-1 constant region.

Human NTR-1 Sequence

```
                10                  20                  30                  40                  50                  60
Seq ID#1  ATG AGG GCG CTG GAG GGG CCA GGC CTG TCG CTG CTG TGC CTG GTG TTG GCG CTG CCT GCC
Seq ID#2  Met Arg Ala Leu Glu Gly Pro Gly Leu Ser Leu Leu Cys Leu Val Leu Ala Leu Pro Ala

                70                  80                  90                  100                 110                 120
          CTG CTG CCG GTG CCG GCT GTA CGC GGA GTG GCA GAA ACA CCC ACC TAC CCC TGG CGG GAC
          Leu Leu Pro Val Pro Ala Val Arg Gly Val Ala Glu Thr Pro Thr Tyr Pro Trp Arg Asp

                130                 140                 150                 160                 170                 180
          GCA GAG ACA GGG GAG CGG CTG GTG TGC GCC CAG TGC CCC CCA GGC ACC TTT GTG CAG CGG
          Ala Glu Thr Gly Glu Arg Leu Val Cys Ala Gln Cys Pro Pro Gly Thr Phe Val Gln Arg

                190                 200                 210                 220                 230                 240
          CCG TGC CGC CGA GAC AGC CCC ACG ACG TGT GGC CCG TGT CCA CCG CGC CAC TAC ACG CAG
          Pro Cys Arg Arg Asp Ser Pro Thr Thr Cys Gly Pro Cys Pro Pro Arg His Tyr Thr Gln

                250                 260                 270                 280                 290                 300
          TTC TGG AAC TAC CTG GAG CGC TGC CGC TAC TGC AAC GTC CTC TGC GGG GAG CGT GAG GAG
          Phe Trp Asn Tyr Leu Glu Arg Cys Arg Tyr Cys Asn Val Leu Cys Gly Glu Arg Glu Glu

                310                 320                 330                 340                 350                 360
          GAG GCA CGG GCT TGC CAC GCC ACC CAC AAC CGT GCC TGC CGC TGC CGC ACC GGC TTC TTC
          Glu Ala Arg Ala Cys His Ala Thr His Asn Arg Ala Cys Arg Cys Arg Thr Gly Phe Phe

                370                 380                 390                 400                 410                 420
          GCG CAC GCT GGT TTC TGC TTG GAG CAC GCA TCG TGT CCA CCT GGT GCC GGC GTG ATT GCC
          Ala His Ala Gly Phe Cys Leu Glu His Ala Ser Cys Pro Pro Gly Ala Gly Val Ile Ala

                430                 440                 450                 460                 470                 480
          CCG GGC ACC CCC AGC CAG AAC ACG CAG TGC CAG CCG TGC CCC CCA GGC ACC TTC TCA GCC
          Pro Gly Thr Pro Ser Gln Asn Thr Gln Cys Gln Pro Cys Pro Pro Gly Thr Phe Ser Ala

                490                 500                 510                 520                 530                 540
          AGC AGC TCC AGC TCA GAG CAG TGC CAG CCC CAC CGC AAC TGC ACG GCC CTG GGC CTG GCC
          Ser Ser Ser Ser Ser Glu Gln Cys Gln Pro His Arg Asn Cys Thr Ala Leu Gly Leu Ala

                550                 560                 570                 580                 590                 600
          CTC AAT GTG CCA GGC TCT TCC TCC CAT GAC ACC CTG TGC ACC AGC TGC ACT GGC TTC CCC
          Leu Asn Val Pro Gly Ser Ser Ser His Asp Thr Leu Cys Thr Ser Cys Thr Gly Phe Pro

                610                 620                 630                 640                 650                 660
          CTC AGC ACC AGG GTA CCA GGA GCT GAG GAG TGT GAG CGT GCC GTC ATC GAC TTT GTG GCT
          Leu Ser Thr Arg Val Pro Gly Ala Glu Glu Cys Glu Arg Ala Val Ile Asp Phe Val Ala

                670                 680                 690                 700                 710                 720
          TTC CAG GAC ATC TCC ATC AAG AGG CTG CAG CGG CTG CTG CAG GCC CTC GAG GCC CCG GAG
          Phe Gln Asp Ile Ser Ile Lys Arg Leu Gln Arg Leu Leu Gln Ala Leu Glu Ala Pro Glu

                730                 740                 750                 760                 770                 780
          GGC TGG GGT CCG ACA CCA AGG GCG GGC CGC GCG GCC TTG CAG CTG AAG CTG CGT CGG CGG
          Gly Trp Gly Pro Thr Pro Arg Ala Gly Arg Ala Ala Leu Gln Leu Lys Leu Arg Arg Arg

                790                 800                 810                 820                 830                 840
          CTC ACG GAG CTC CTG GGG GCG CAG GAC GGG GCG CTG CTG GTG CGG CTG CTG CAG GCG CTG
          Leu Thr Glu Leu Leu Gly Ala Gln Asp Gly Ala Leu Leu Val Arg Leu Leu Gln Ala Leu

                850                 860                 870                 880                 890                 900
          CGC GTG GCC AGG ATG CCC GGG CTG GAG CGG AGC GTC CGT GAG CGC TTC CTC CCT GTG CAC
          Arg Val Ala Arg Met Pro Gly Leu Glu Arg Ser Val Arg Glu Arg Phe Leu Pro Val His

          TGA
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(54) **Title:** HUMAN ORPHAN RECEPTOR NTR-1

(57) **Abstract**

The present invention provides for a nucleic acid sequence, designated as HUMAN NTR-1, that encodes a novel orphan receptor expressed in pancreas and fetal heart. The invention also provides assay systems that may be used to detect and/or measure ligands that bind the HUMAN NTR-1 gene product. The present invention also provides for diagnostic and therapeutic methods based on the interaction between NTR-1 and agents that initiate signal transduction through binding to NTR-1.

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 98/16202

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N15/62 C12N15/63
C12P21/08 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER L ET AL: "Human cDNA clone 366305" EMBL SEQUENCE DATABASE,16 August 1996, XP002091562 HEIDELBERG DE cited in the application Accession Nr.: AA025673 see abstract ---	1-5
X	HILLIER L ET AL: "Human cDNA clone 592256" EMBL SEQUENCE DATABASE,18 January 1997, XP002091560 HEIDELBERG DE cited in the application Accession Nr.: AA155646 see abstract --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1999	12/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

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INTERNATIONAL SEARCH REPORT

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PCT/US 98/16202

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DE 196 54 610 A (AMGEN INC) 26 June 1997 cited in the application see page 1 - page 13	1-18
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E	EP 0 861 850 A (SMITHKLINE BEECHAM CORP) 2 September 1998 see page 1, line 1 - page 2, line 16; figure 1 see page 4, line 50 - page 10, line 55	1-16

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INTERNATIONAL SEARCH REPORT

In...ational application No.
PCT/US 98/ 16202

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

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☐ The additional search fees were accompanied by the applicant's protest.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
PCT/US 98/16202

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